

The adrenocortical response of tufted puffin chicks to nutritional deficits

Alexander S. Kitaysky^{a,*}, Marc D. Romano^b, John F. Piatt^b,
John C. Wingfield^c, Motoshi Kikuchi^d

^a*Institute of Arctic Biology, 311 Irving I, University of Alaska Fairbanks, Fairbanks, AK 99775, USA*

^b*Alaska Biological Science Center, USGS, 1011 East Tudor Road, Anchorage, AK 99503, USA*

^c*Department of Biology, University of Washington, Box 351800, Seattle, WA 98195, USA*

^d*Division of Histology and Cell Biology, Department of Anatomy, Jichi Medical School Yakushiji 3311-1, Minamikawachi-machi, Tochigi 329-0498, Japan*

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Abstract

In several seabirds, nutritional state of a nest-bound chick is negatively correlated with the activity of its hypothalamus–pituitary–adrenal (HPA) axis. Increased corticosterone (cort) secretion has been shown to facilitate changes in behavior that allow hungry chicks to obtain more food from parents. However, if parents are not willing/able to buffer their young from temporary food shortages, increased cort secretion could be detrimental to undernourished chicks. In a system where parents are insensitive to chick demands, low benefits and high costs of activation of the HPA-axis in hungry chicks should lead to a disassociation of the nutritional state of the young and the activity of its HPA-axis. We tested this novel hypothesis for the tufted puffin (*Fratercula cirrhata*), a seabird with intermittent provisioning of a nest-bound semi-precocial chick. We examined the HPA-axis activity of captive chicks exposed to the following: (1) a short-term (24 h) food deprivation; and (2) an array of prolonged (3 weeks) restrictions in feeding regimens. We found that in response to a short-term food deprivation chicks decreased baseline levels of cort and thyroid hormones. In response to prolonged restrictions, food-limited chicks exhibited signs of nutritional deficit: they had lower body mass, endogenous lipid reserves, and thyroid hormone titers compared to chicks fed ad libitum. However, baseline and maximum acute stress-induced levels of cort were also lower in food-restricted chicks compared to those of chicks fed ad libitum. These results support a major prediction of the study hypothesis that puffin chicks suppress HPA-axis activity in response to short- and long-term nutritional deficits. This physiological adaptation may allow a chick to extend its development in the nest, while eluding detrimental effects of chronic cort elevation.

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Introduction

Adrenocortical responses of chicks to a nutritional challenge are not uniform among avian species. In several seabirds that provision their chicks with food in a continuous manner, the nutritional state of a nest-bound chick is negatively correlated with the activity of its hypothalamus–pituitary–adrenal (HPA) axis. Elevated levels of corticosterone (cort) in chicks were documented within

24–48 h of fasting in the glaucous-winged gull x Western gull hybrid *Larus glaucescens* x *L. occidentalis* (A. Edwards, unpublished data) and in the blue-footed booby *Sula nebouxii* (Nunez-de la Mora et al., 1996), during several weeks of moderate food restriction in black-legged *Rissa trydactyla* and red-legged *Rissa brevirostris* kittiwakes (Kitaysky et al., 1999, 2001a), and in undernourished Magellanic penguins *Spheniscus magellanicus* (Walker et al., in press). In these studies, increased cort secretion was associated with depleted endogenous fat reserves of a chick, and high titers of cort could be required to alter metabolic pathways so that nutritionally stressed individuals can rely on catabolism of proteins to fuel their activities (Axelrod

* Corresponding author. Fax: +1 907 474 6967.

E-mail address: ffask@uaf.edu (A.S. Kitaysky).

and Reisine, 1984; Cherel et al., 1992; Le Ninan et al., 1988). Other studies did not find an increase in cort secretion in response to low nutritional condition and/or reduction in energy intake in chicks and juveniles of three species of passerines (Romero et al., 1998; Schwabl, 1999; Sims and Holberton, 2000), young domestic fowls (*Gallus gallus domesticus*, Rees et al., 1985), domestic turkey (*Meleagris gallopavo*, in vitro study by Carsia and McIlroy, 1998), and American kestrels (*Falco sparverius*, Heath and Dufty, 1998). Potentially, different combinations of life history, phylogenetic, and ecological factors determine whether the HPA-axis of chicks is activated or suppressed in response to a nutritional challenge (Kitaysky et al., 2003).

It might be beneficial for nest-bound chicks of some species to suppress activity of HPA-axis during food shortages. Increased cort secretion in chicks has been shown to increase parental food provisioning rates which may restore homeostasis of hungry chicks (Kitaysky et al., 2001b). However, parents are not always willing (or able) to compensate for a decrease in food availability by increasing their effort in foraging for the young, and chick provisioning with food may remain low for extended periods of time (e.g., Harding et al., 2002; Kitaysky, 1996; Kitaysky et al., 2000; Piatt and Kitaysky, 2002a,b; Ricklefs, 1987, 1992; Takahshi et al., 1999). Specifically, chick-provisioning rates may be altered in the following: (a) frequency of food delivery, (b) quantity of food, and (c) quality of food. When these are the cases, some seabird chicks are able to adjust their developmental rate to the rate of energy provisioning by parents, extend the duration of their development in the nest, and fledge successfully despite severe food shortages (e.g., Hamer and Hill, 1994; Kitaysky, 1999). In mammals, if cort secretion remains elevated during a slow-development period, it can be detrimental to affected individuals (Sapolsky, 1992; Sapolsky et al., 1986). In birds, individuals can suffer decreased growth efficiency and permanent impairment of their cognitive function if exposed to even a short episode of elevated corticosterone levels during development (Kitaysky et al., 2003). It is not known whether suppressed HPA-axis activity is characteristic for seabird chicks that are capable of slowing development in response to nutritional deficits. As a short-term benefit (on a scale of hours to days), reduced cort production would allow under-nourished seabird chicks to conserve endogenous lipid reserves and avoid loss of skeletal muscle proteins (reviewed in Sapolsky et al., 2000). As a long-term benefit, reduced cort production would allow slowly developing individuals to elude lasting detrimental effects of chronically increased cort. Therefore, depending on environmental stochasticity (Ricklefs and Schew, 1994; Wingfield and Kitaysky, 2002) and parental responsiveness to a chick's demands, selection may favor a disassociation of the nutritional state of the young and the activity of its HPA-axis in species where nest-bound chicks routinely experience intermittent food provisioning.

In this study we tested this novel hypothesis for the tufted puffin (*Fratercula cirrhata*), a seabird with intermittent provisioning of a single nest-bound semi-precocial chick (Piatt and Kitaysky, 2002a). The major goal of this study was to assess the activity of the HPA-axis (baseline levels of cort and standardized acute stress-response [*sensu*; Wingfield et al., 1992]) of chicks experimentally exposed to nutritional deficits. Our first objective was to test a chick's response to a short-term food deprivation. Our second objective was to test a chick's response to a wide range of prolonged restrictions in feeding regimens. Specifically we tested three likely scenarios of alterations in chick food intake: (a) frequency of energy delivery, (b) prolonged restriction in quantity of energy, and (c) prolonged restriction in quality of food. The results of recent experimental studies of kittiwakes show that baseline and acute stress induced levels of cort increase in a continuous manner with the severity of dietary restriction and depletion of endogenous fat reserves of individuals (Kitaysky et al., 1999, 2001a). However, several other studies reported a threshold function relating the activity of HPA-axis to the nutritional state of animals, suggesting that cort secretion would not increase until animals nearly depleted their endogenous fat reserves and were forced switch to structural proteins as an alternative energy substrate (Cherel et al., 1988; Le Ninan et al., 1988; Romero and Wikelski, 2000). Thus, it is important to examine the adrenocortical response of chicks to a wide range of nutritional limitations in order to detect the general shape of the response to an experimental treatment. Controlled experiments have shown that food-related stress can account for low body mass, depleted fat reserves, and changes in titers of thyroid hormones (e.g., Boag, 1987; Geris et al., 1999; Kitaysky, 1996; Romano, 2000; Schew et al., 1996). These were the focal parameters we measured in addition to the measurements of diet-induced changes in HPA-axis. For comparison with a natural system, we also measured endocrinological parameters of wild tufted puffin chicks.

Materials and methods

Free-living tufted puffin chicks (ages ranged from 4 to 18 days post-hatch) were taken from their nests on the Barren Islands, Alaska, kept in captivity (at Kasitsna Bay Research Laboratories, University of Alaska Fairbanks) in individual nest boxes, and fed a mixture of forage fish given ad libitum until experiments. All chicks were kept under thermoneutral conditions and natural photoperiod, and food was provided in dishes placed on the bottom of nest boxes. Two experiments were conducted.

The first experiment was designed to examine endocrine responses of chicks to a short-term food deprivation. Prior to the experiment, 21 chicks were offered a mix of high quality fish ad libitum four times a day. Then individuals were randomly assigned to two treatments. In the "food deprived"

group, individuals were not fed for 24 h. Controls remained on the ad libitum feeding regime. All chicks were housed in the same room and exposed to the same husbandry events. All chicks were bled on the same schedule. The first blood sample of undisturbed (collected immediately after taking a chick from the nest) post-absorptive (after 12-h overnight fast) chicks was taken at the beginning of the experiment. The second blood sample was taken 12 h later so that individuals in a food-deprived group were fasting for 24 h, while controls were post-absorptive (3 h since last feeding). Blood samples were collected and processed as described below.

The second experiment was designed to assess endocrine responses of chicks to prolonged restrictions in quality and quantity of the diet and feeding frequency (Table 1). 41 individuals, fed ad libitum until the experiment, were randomly assigned to seven treatments. The diet quality was assessed based on (1) the lipid to protein ratio, LPR, determined via proximate analyses of whole fish (for details, see Kitaysky et al., 1999; Romano, 2000), and (2) the apparent metabolizable energy (or digestible energy) of fish, measured as the energy value of food minus the energy value of feces (Romano, 2000). Starting at the age of 3 weeks post-hatch, either low lipid/digestibility fish (thereafter called “low-quality diet”), walleye pollock, *Theragra chalcogramma* (lipid to protein ratio, LPR = 0.3–0.4; digestibility 72%), or high lipid/digestibility (thereafter called “high-quality diet”) capelin, *Mallotus villosus* (LPR = 1.26; digestibility 88%), and Pacific herring, *Chupea harengus* (LPR = 1.47; digestibility 88%), were fed to chicks for 3 weeks (Table 1). Quantities of fish fed to chicks in different treatments were determined as following. In a previous study (Kitaysky, 1999), food intake of 20–40 days old tufted puffin chicks fed ad libitum with a mix of forage fish, which included capelin, was measured at 132.5 ± 19.04 (SD) g day⁻¹ (wet mass). Assuming that energy content of food was similar to the metabolizable energy content of capelin in this study, tufted puffin chicks fed ad libitum consumed 715.8 ± 102.9 (SD) kJ day⁻¹ during 20–40 days post-hatch. Therefore, in this study chick energy intake was reduced to approximately 30%, 60%, and 100% of the ad libitum ration (Table 1).

All chicks were fed four meals daily, except for one treatment where chicks were fed in a similar manner but every other day (Table 1). Specifically, in this treatment chicks were fed 116 g of herring given every other day, so that the total amount of energy received during the 3-week experiment would be the same as for the chicks in the “58 g day⁻¹ herring” treatment. The main purpose of this treatment was to examine sensitivity of puffin chicks to feeding frequency vs. the total amount of metabolizable energy provided during post-embryonic development.

To control for possible effects of captivity on the activity of HPA-axis of experimental chicks, free-living chicks of approximately the same age were exposed to the same handling stress protocol, and blood samples were taken as described below. Exact age of wild chicks was not known; comparisons of the wing chord development between two captive treatments fed approximately ad libitum and wild chicks suggest that wild chicks could have been somewhat older than the captive chicks (Table 1). Diet composition of wild chicks was also not known, but adults observed at the same colony were delivering meals that consisted of high lipid fish: Pacific sandlance, *Ammodytes hexapterus*, herring, juvenile salmon, and capelin (authors’ personal observations).

A blood sample of undisturbed (collected immediately after taking a chick from the nest) post-absorptive (after overnight fast) chicks was taken at the beginning and the end of the experiment. Blood samples were collected by puncturing the alar vein and collecting blood in 100 µl heparinized hematocrit tubes. At the end of the experiment, post-absorptive chicks were exposed to a standardized acute handling and restraint stress protocol, where in addition to the first blood sample (baseline) three blood samples (acute stress response) were taken at intervals of 10, 30, and 50 min after capture. All captive and wild chicks were bled between 10.00 and 14.00 h according to this standardized protocol. After each sample, blood flow was stopped by the application of cotton and birds were placed individually into open plastic buckets. After blood collection, hematocrit tubes were emptied into 0.5 ml vials, which were stored on ice (in the

Table 1
Experimental protocols, body mass, and wing chord of tufted puffin chicks (LPR represents lipid to protein ratio)

Diet composition	LPR	Food intake (g day ⁻¹ , wet mass)	Energy intake (kJ day ⁻¹)	Change in body mass (g, wet mass)		Body mass at 6 weeks (g, wet mass)		Wing chord at 6 weeks (mm)		
				Mean	SE	Mean	SE	Mean	SE	n
Walleye pollock	0.30	80	213.1	92.4	8.32	313.8	8.94	129.6	2.29	5
Capelin	1.26	45	243.6	72.7	5.94	295.4	4.10	131.0	1.45	7
Pacific herring	1.47	58	366.1	179.2	5.54	395.5	1.96	137.8	1.14	6
Pacific herring	1.47	58 ^a	366.1	161.8	7.75	404.2	3.38	130.8	0.38	5
Walleye pollock	0.41	100	379.4	238.2	10.04	473.7	4.62	140.5	1.96	6
Pacific herring	1.47	100	631.3	326.3	15.89	563.3	10.67	142.7	1.86	6
Walleye pollock	0.41	172	652.6	378.0	9.39	641.2	7.29	144.7	0.71	6
Unknown ^b						590.0	16.51	149.4	3.63	5

^a Fed 116 g every other day.

^b ~6 weeks old wild chicks.

field) or in a refrigerator at 4°C (in the laboratory). Blood samples were centrifuged and plasma collected within 6 h.

Plasma samples were frozen at –20°C until radioimmunoassay analyses (for details, see Wingfield and Farner, 1975; Wingfield et al., 1992). The concentrations of corticosterone in plasma samples were measured from 20 to 30 µl of plasma. Steroid concentrations were measured in duplicate for each sample after extraction in 4 ml of dichloromethane. Recovery values (ranging from 90% to 97%) following extraction were used to adjust assayed concentrations of steroids. All baseline plasma samples were processed in one assay, and all plasma samples collected during stress-series were randomly assigned and processed in two additional assays. Intra- and inter-assay coefficients of variation were 4% and 7%, respectively.

Thyroid hormone analyses were performed at the Jichi Medical School, Tochigi, Japan. The concentrations of total thyroxin (T4) and 3',5,3-triiodothyronine (T3) in 10 and 25 µl, for T4 and T3, respectively, of plasma samples (collected immediately after capture) were measured directly (no extraction involved) in duplicates using a commercially available kit and an automatic enzyme immunoassay instrument (AIA-600) from Tosho Corp. Japan (details of the assay are available at <http://www.tosoh.com/EnglishHomePage/tcdiv/ftrcdiv.htm>). The immunoassay was validated for determining T3 and T4 in avian plasma, and we followed the same protocol, using dextran-charcoal stripped Japanese quail (*Coturnix coturnix*) plasma as a diluent for the standard hormone and samples (S. Ishii and M. Kikuchi, Waseda University, Tokyo, Japan, personal communication). Except for this, we followed the manufacturers' instructions. The intra- and inter-assay coefficients of variation were 2.3–6.1% and 3.7–6.8% for T3 and T4, respectively. Sensitivities of the assays were 0.18 ng ml⁻¹ and 0.5 µg dl⁻¹ for T3 and T4, respectively.

At the age of 40 days post-hatch (the earliest possible fledging age; Wehle, 1980), the experimental birds were anesthetized with diethyl ether and sacrificed via cervical dislocation. Carcasses were frozen at –20°C until proximate analyses of the whole body were conducted (for details, see Romano, 2000). Moisture content was determined by air-drying plucked carcasses to a constant mass at +60°C in a forced convection oven. Total body lipids were determined by the extraction of aliquots of dried homogenate in a Soxhlet apparatus with petroleum ether as a solvent. We used total body lipids as a measure of endogenous energy reserves (assuming energy equivalent of lipids at 39 kJ day⁻¹; Schmidt-Nielsen, 1997).

Statistical analyses

The effects of the short-term food deprivation on baseline levels of hormones were examined with repeated-measures ANOVA (followed by critical range post hoc tests), where experimental treatments were used as factors and samples obtained from the same chick before and after the treatment

as repeated measures. The effects of the long-term food restrictions on baseline levels of cort and T4 were examined with repeated-measures ANOVA (followed by critical range post hoc tests), where experimental treatments were used as a factor. Because T3 levels in the most restricted treatments were below the low level of detectability of our assay, repeated-measures ANOVA design was not appropriate statistical test to use (see below). Thus we used a regular ANOVA to test for differences in T3 levels at 3 weeks (beginning of the experiment) and Kruskal–Wallis non-parametric ANOVA to test for differences in T3 levels at 6 weeks (end of the experiment; all undetectable values were set at the level of detectability, 0.18 ng ml⁻¹). Changes in the T3/T4 ratio during experiments were examined with the non-parametric median test, with treatments as factor and a relative change in T3/T4 ratio, calculated as [(Ratio_{6 weeks} – Ratio_{3 weeks}) / Ratio_{3 weeks}], as a dependent variable. Differences in the adrenocortical response to acute stress of handling were examined with repeated-measures ANOVA, where treatments were used as a factor and concentrations of cort, at 0, 10, 30, and 50 min of the acute stress of handling, as repeated measures. The effect of diet on maximum concentrations of cort during acute stress of handling (which occurred at 10, 30, or 50 min of handling, hereafter called “maximum”), was examined with a repeated-measures ANOVA (followed by critical range post hoc tests), with diet as factor and baseline and maximum cort as repeated measures. The relationship between baseline and maximum concentrations of cort, the effects of chick's body lipids on baseline and maximum levels of cort, and the relationship between body mass and water content were examined by using regression analyses. The effects of diet quality vs. daily energy intake on circulating levels of corticosterone were examined with ANCOVA, where diet quality (low vs. high, as identified above) was used as a factor and daily energy intake as a covariate.

For statistical analyses, initial data were tested for assumptions required by parametric statistical tests according to Sokal and Rohlf (1981). If these data violated assumptions they were ln/log₁₀-transformed and examined again. If the transformed data still violated assumptions, non-parametric statistical tests were performed. All computations were performed by using Statistica and SYSTAT statistical packages. Statistical significance was assumed at $P < 0.05$.

Manipulations with birds were conducted according to the rules of IACUC of the University of Alaska Fairbanks, Oregon State University, and University of Washington, and under the federal and state collection permits.

Results

Endocrine responses to a short-term food deprivation

Body mass (208.6 ± 6.0 (SE) g and 220.7 ± 3.57 (SE) g, for fasting and control groups, respectively) and age

(16.9 ± 1.24 (SE) days and 19.0 ± 1.01 (SE) days, for fasting and control groups, respectively) were similar between treatments at the beginning of the experiment.

24-h food deprivation affected baseline levels of cort in fasting chicks compared to controls (repeated-measures ANOVA on ln-transformed data: treatment \times duration of the experiment, $F_{1,19} = 5.511$, $P = 0.029$; Fig. 1A). At the beginning and the end of experiment, baseline levels of cort were not significantly different between treatments (Fig. 1A). However, at the end of the 24-h period, baseline levels decreased ($P = 0.019$) by 10.2% in fasting chicks but did not change in controls (Fig. 1A).

24-h food deprivation decreased plasma concentrations of T4 in fasting chicks compared to controls (repeated-measures ANOVA on \log_{10} -transformed data: treatment \times duration of the experiment, $F_{1,19} = 16.94$, $P < 0.001$; Fig. 1B). T4 concentrations were not significantly different between treatments at the beginning of experiment, but they were lower in fasting chicks compared to controls at the end of experiment ($P = 0.029$). During a 24-h period, fasting decreased T4 levels in experimental chicks ($P < 0.001$), but T4 levels did not change in controls (Fig. 1B).

Similarly, T3 levels decreased in fasting chicks but did not change in controls (repeated-measures ANOVA on ln-transformed data: treatment \times duration of the experiment, $F_{1,19} = 6.56$, $P = 0.019$; Fig. 1C). T3 concentrations were not different between treatments at the beginning of experiment but were lower in fasting chicks compared to controls at the end of experiment ($P = 0.023$). More importantly, during 24 h T3 levels decreased in fasting chicks ($P < 0.001$) but did not change in controls (Fig. 1C).

T3/T4 ratio did not change significantly between the beginning and the end of experiment and was similar between fasting chicks and controls (median test, chi-square = 0.043, $df = 1$, $P = 0.835$).

Endocrine responses to long-term dietary restrictions

Effects of nutritional challenge on baseline levels of cort

Baseline levels of cort differed among treatments (repeated-measures ANOVA on ln-transformed data, diet effect: $F_{6,34} = 2.503$, $P = 0.041$; Fig. 2). Baseline levels of cort changed during 3 weeks dietary restrictions (treatment duration effect: $F_{1,34} = 20.474$, $P < 0.001$; Fig. 2). Yet, there was a significant interaction between diet and treatment duration ($F_{6,34} = 2.48$, $P = 0.043$) because chicks in the two most restricted treatments decreased their baseline levels of cort, but chicks in all other treatments did not (Fig. 2).

Acute stress-induced levels of cort

Diet affected the adrenocortical response of chicks to a standardized acute stress of capture and restraint (repeated-measures ANOVA on \log_{10} -transformed data, $F_{6,34} = 2.63$, $P < 0.04$). Handling increased secretion of cort

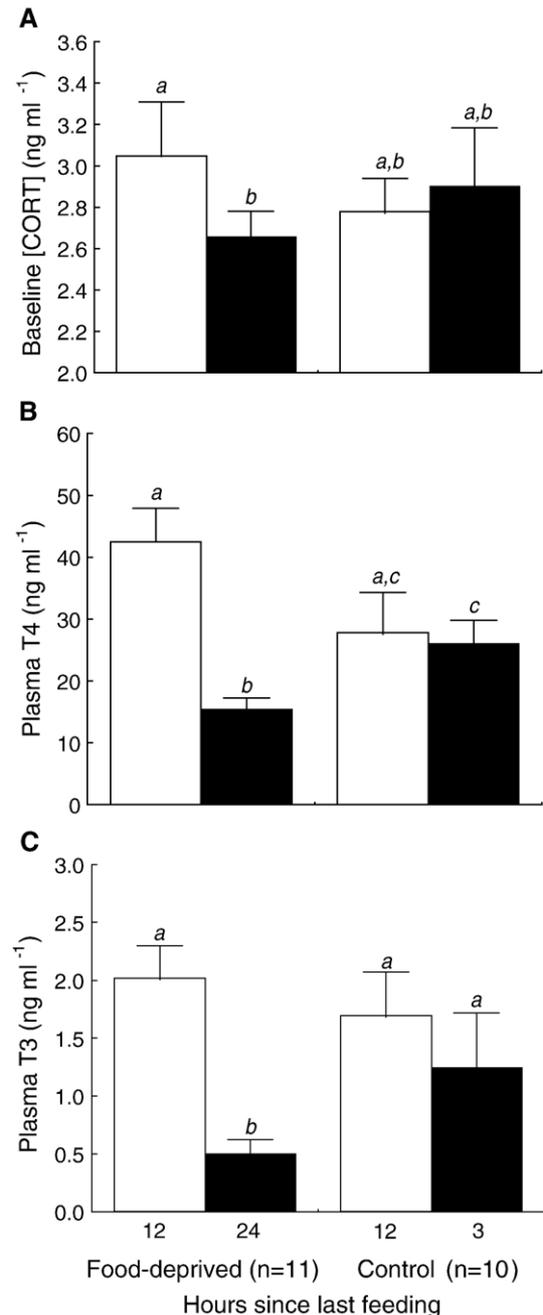


Fig. 1. Effects of short-term food deprivation on concentrations of cort (panel A), T4 (panel B), and T3 (panel C) in tufted puffin chicks (mean + SE). Bars represent measurements of hormones at the beginning (white bars) and the end (black bars) of experiment. Hours since last feeding, treatments and sample sizes are shown in the lower portion of panel C. Letters above SE bars represent post hoc statistical comparisons between and within treatments, common letters indicate no difference and different letters indicate significant differences.

($F_{3,102} = 17.79$, $P < 0.001$), yet the rate of increase in cort secretion was different among treatments (diet \times handling interaction term: $F_{18,102} = 2.74$, $P < 0.001$). Diet also affected the maximum levels of cort achieved by chicks during a standardized acute stress of capture and restraint (repeated-measures ANOVA on \log_{10} -transformed

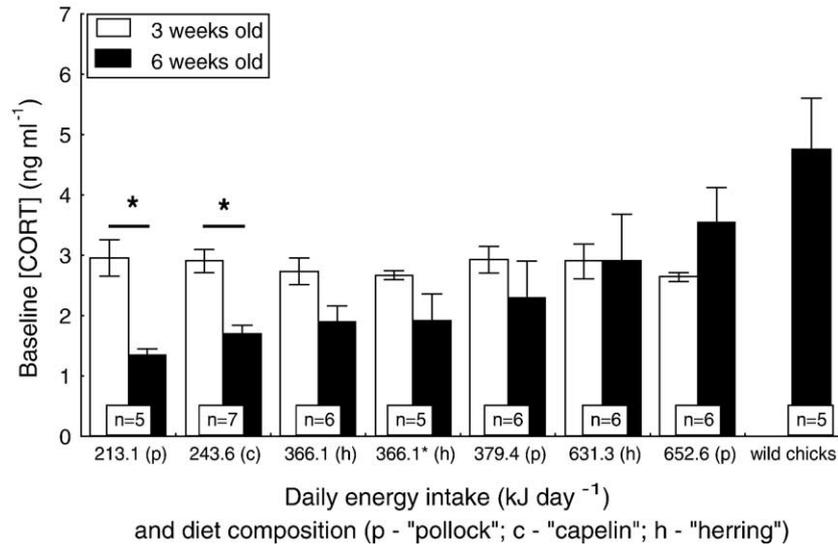


Fig. 2. Effects of long-term nutritional deficit on baseline levels of cort in captive tufted puffin chicks (means \pm SE). White bars represent measurements at 3 weeks old (before dietary treatments) and black bars represent measurements at 6 weeks old (3 weeks after dietary restrictions started). Asterisks indicate a significant change at $P < 0.05$. Numbers shown in boxes represent sample sizes for different treatments. “366.1* (h)” designates a dietary treatment where chicks were fed 732.2 kJ of herring every other day (see Materials and methods).

data, $F_{6,34} = 2.65$, $P = 0.032$; Fig. 3). Maximum levels were significantly higher in the less restricted treatments when compared to the most restricted treatments (critical range post hoc tests P values ranging between $P < 0.018$ and $P < 0.001$; Fig. 3).

Effects of nutritional challenge on plasma concentrations of thyroid hormones

Plasma levels of T4 differed among treatments (repeated-measures ANOVA on \log_{10} -transformed data, diet effect:

$F_{6,34} = 5.175$, $P < 0.001$; Fig. 4A). There was a significant interaction between diet and treatment duration ($F_{6,34} = 10.879$, $P < 0.001$) because chicks in the two most restricted treatments decreased secretion of T4, but chicks in all other treatments did not (Fig. 4A).

Plasma levels of T3 were similar among treatments at the beginning of experiment (ANOVA on \log_{10} -transformed data, $F_{6,34} = 0.855$, $P = 0.537$; Fig. 4B). At the end of the experiment plasma levels of T3 differ among treatments (Kruskal–Wallis test; $H_{6, N = 41} = 29.33$, $P =$

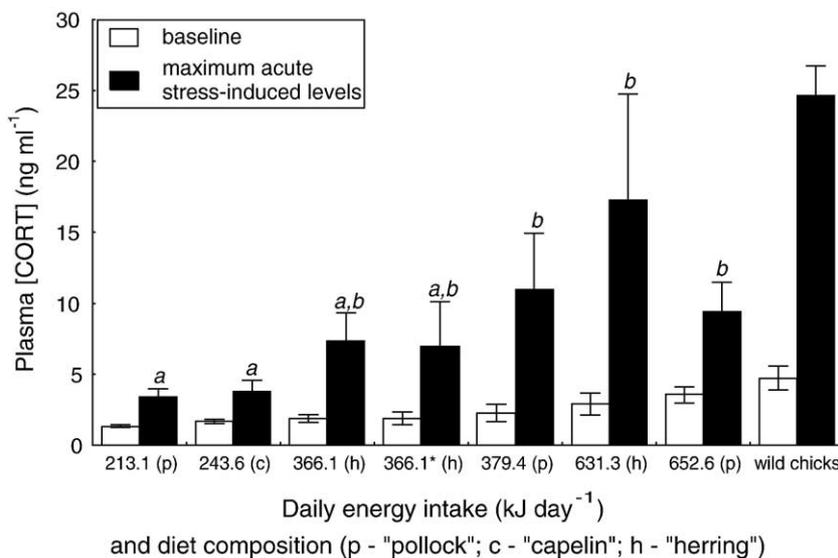


Fig. 3. Effects of long-term nutritional deficit on the adrenocortical response in 6-week-old captive tufted puffin chicks in relation to dietary treatments, and in wild tufted puffin chicks of ages similar to captives (means \pm SE). White bars represent baseline concentrations of cort measurements, and black bars represent maximum concentrations of cort achieved by chicks during acute stress of handling and restraint. Letters above SE bars represent post hoc comparisons of maximum cort among treatments, common letters indicate no difference and different letters indicate statistically significant differences. Sample sizes and treatments are as shown in Fig. 2.

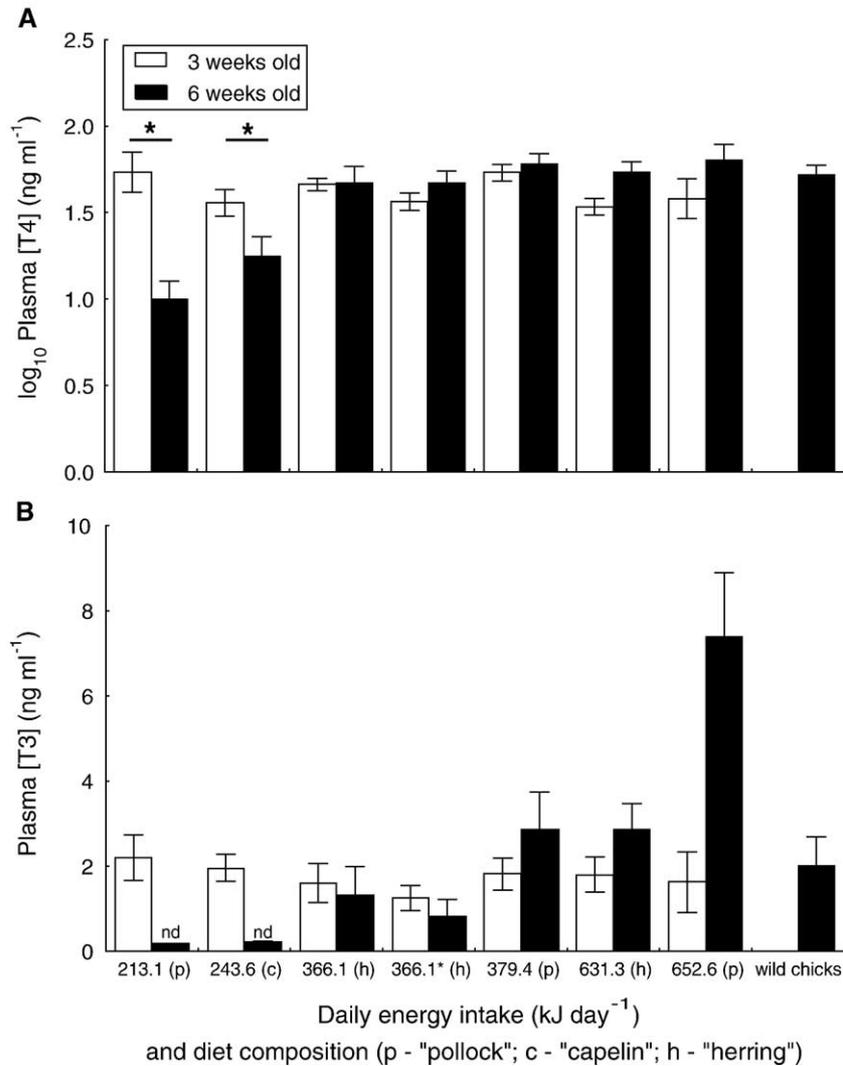


Fig. 4. Effects of long-term nutritional deficit on plasma concentrations of T4 (panel A) and T3 (panel B) in captive 6-week-old tufted puffin chicks in relation to dietary treatments, and in wild 6-week-old tufted puffin chicks (means \pm SE). White bars represent measurements at 3 weeks old (before food restriction started) and black bars represent measurements at 6 weeks old (3 weeks after dietary restrictions started). Asterisks on panel A indicate a significant change in T4 concentrations between the beginning and the end of experiment at $P < 0.05$. See text for statistical analyses of data presented in panel B; "nd" indicates concentrations too low to be detected in the assay. Sample sizes and treatments are as shown in Fig. 2.

0.001; Fig. 4B). Specifically, T3 titers dropped below the level of detectability in the two most restricted treatments but were somewhat uniform and higher in chicks exposed to either moderate dietary restrictions or fed ad libitum high quality food (Fig. 4B). T3 titers increased dramatically in chicks fed ad libitum low quality food (Fig. 4B), suggesting that processing of large amounts of poor quality food (see Table 1) may require additional increase in metabolic activity, facilitated by T3. Overall, T3 concentrations increased with an increase in daily energy intake (linear regression analysis on log₁₀-transformed data, $R^2 = 0.63$, $F_{1,39} = 67.02$, $P < 0.001$).

At the end of the experiment, T3 levels dropped below the level of detectability in treatments fed 70% restricted diets (Fig. 4). This did not allow us to calculate a change in T3/T4 ratio during the experiment in these treatments. In other treatments, T3/T4 ratio did not change significantly during

the experiment (median test, chi-square = 5.51, $df = 4$, $P = 0.239$; Fig. 5).

Effects of feeding frequency on endocrine parameters

Plasma concentrations of cort and thyroid hormones were not different between the treatments where chicks were fed the same restricted diets of high quality fish on different schedules, 366.1 kJ fed daily and 732.2 kJ fed every other day (see Figs. 2–5 and 8).

Endocrine characteristics of wild chicks

Baseline and acute stress-induced levels of cort and T4 titers were at the high end of the ranges of the same parameters in captive chicks (see Figs. 2, 3, and 4A). Plasma levels of T3 in wild chicks were similar to those in chicks in moderate dietary restriction treatments and in chicks fed high quality fish ad libitum (see Fig. 4B).

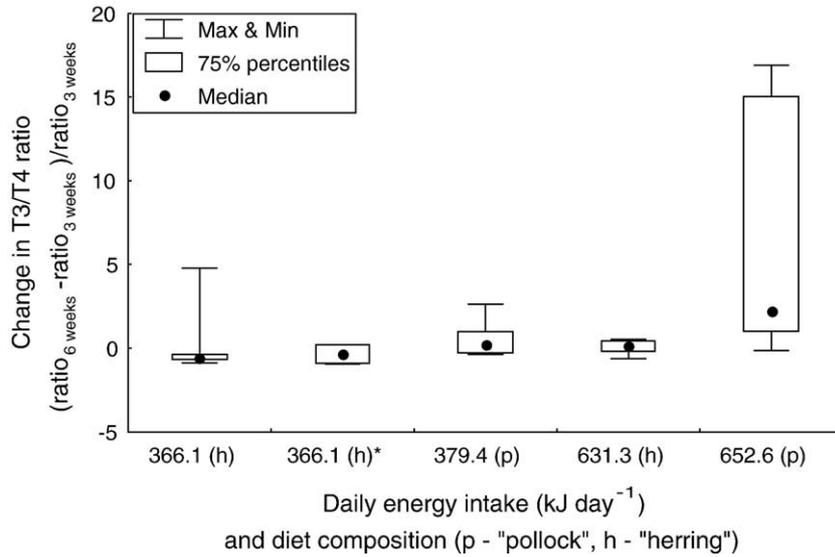


Fig. 5. Effects of long-term nutritional deficit on the change in T3/T4 ratio in captive 6-week-old tufted puffin chicks in response to dietary treatments (medians, min and max, 75% percentiles). Sample sizes and treatments are as shown in Fig. 2.

Circulating levels of cort and body composition

At the end of the experiment, baseline and maximum acute stress-induced levels of cort were significantly positively correlated ($R^2 = 0.41$, $F_{1,44} = 31.04$, $P < 0.001$; Fig. 6). Among the captive chicks the baseline and maximal acute stress-induced levels of cort were significantly positively correlated with body lipids ($R^2 = 0.33$, $F_{1,38} = 18.3$, $P < 0.001$; and $R^2 = 0.20$, $F_{1,38} = 9.24$, $P = 0.004$, for the baseline and maximal levels, respectively; Fig. 7). Food-restricted chicks had lower body mass compared to chicks fed ad libitum (Table 1). However, they also retained more

body water compared to ad libitum fed individuals (diet effect, $F_{6,33} = 85.16$, $P < 0.001$; Fig. 8).

Circulating levels of cort and diet quality vs. daily energy intake

The slopes of relationships between daily energy intake and baseline levels of cort were statistically indistinguishable between groups fed low and high quality food (test for homogeneity of slopes: $F_{1,37} = 1.632$, $P = 0.209$). The daily energy intake had a significant positive effect on baseline levels of cort (ANCOVA on \log_{10} -transformed

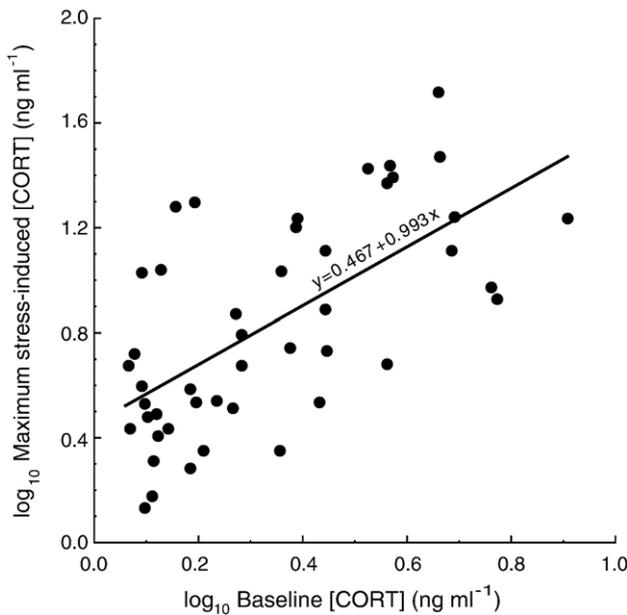


Fig. 6. The relationship between baseline and maximum acute stress-induced levels of cort in 6-week-old tufted puffin chicks. The equation and line represent linear regression analysis.

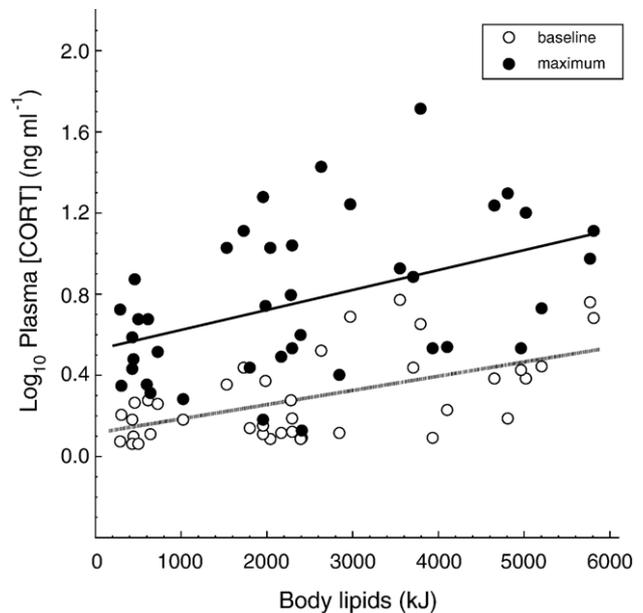


Fig. 7. The relationship between endogenous fat reserves and baseline (open circles, dashed line) and maximum acute stress-induced levels of cort (black circles, solid line) in 6-week-old captive tufted puffin chicks.

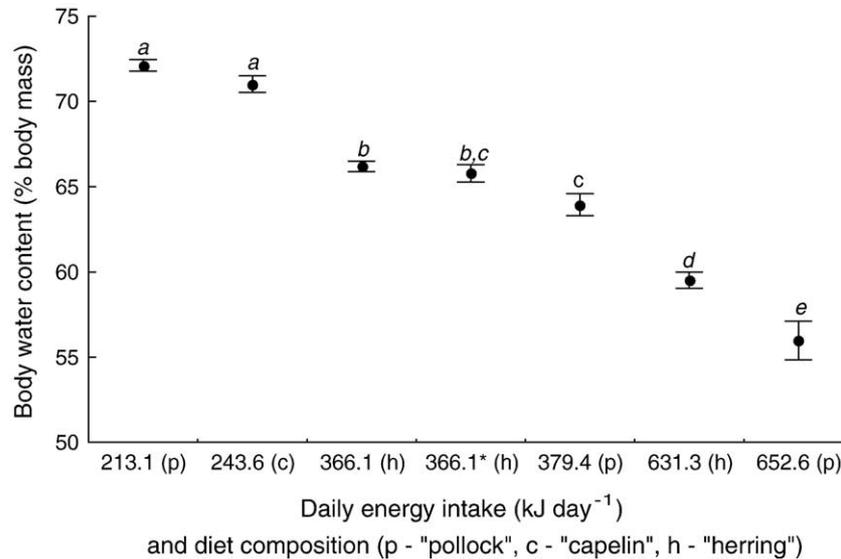


Fig. 8. The effect of diet composition on water retention in 6-week-old captive tufted puffin chicks (means \pm SE). Letters above SE bars represent post hoc comparisons among treatments, common letters indicate no difference, and different letters indicate statistically significant differences ($P < 0.05$). Sample sizes and treatments are as shown in Fig. 2.

data, energy intake as a covariate: $F_{1,38} = 15.52$, $P < 0.001$; Fig. 9), but quality of food controlled for energy content did not (quality as a factor: $F_{1,38} = 0.281$, $P = 0.599$; Fig. 9).

The slopes of relationships between daily energy intake and maximum acute stress-induced levels of corticosterone were statistically indistinguishable between groups fed low and high quality food (test for homogeneity of slopes:

$F_{1,37} = 0.559$, $P = 0.459$). The daily energy intake had a significant positive effect on maximum levels of cort (ANCOVA on \log_{10} -transformed data, energy intake as a covariate: $F_{1,38} = 9.84$, $P = 0.003$; Fig. 9), but quality of food controlled for its energy content did not ($F_{1,38} = 0.007$, $P = 0.936$; Fig. 9).

Discussion

In this study we tested the hypothesis that in a system where nest-bound chicks routinely experience intermittent food provisioning, selection may favor a disassociation of the nutritional state of the young and the activity of its HPA-axis. We presented a detailed description of the endocrinological parameters of tufted puffin chicks growing under conditions of nutritional deficits. These parameters might be used to assess nutritional deficits in free-living chicks of the tufted puffin, the species that has been proposed as one of the model marine top predators for monitoring environmental changes in the North Pacific. We examined the adrenocortical response of tufted puffin chicks to a short-term food deprivation and long-term experimental variations in energy content and quality of food. As predicted by our study hypothesis, we found that tufted puffin chicks do not change baseline and acute-stress-induced levels of cort in response to moderate dietary restrictions in feeding frequency, daily energy intake, or quality of food. In particular, we found that (1) cort levels did not change in response to a reduction in energy intake by 40% of ad libitum regime or in response to the altered feeding schedule (fed every day or every other day); (2) cort levels did not change in response to alterations in food quality adjusted for metabolizable energy content. We also found that tufted puffin chicks

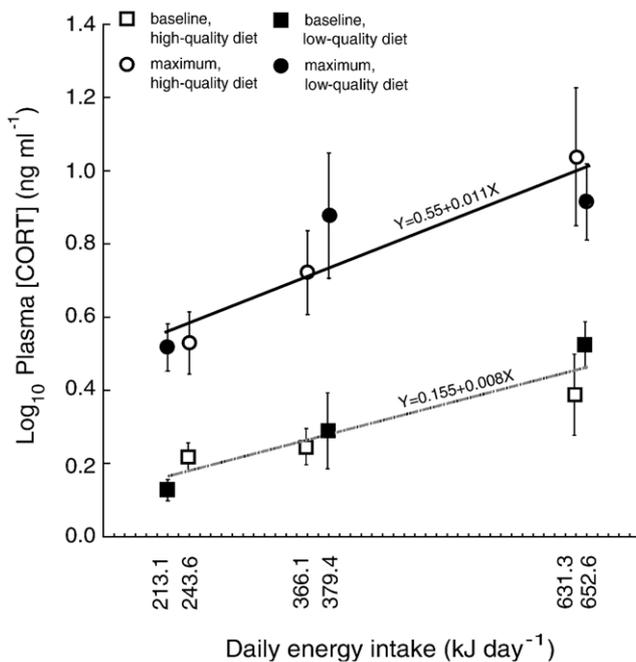


Fig. 9. Effects of daily energy intake and food quality on baseline (shown as squares, dashed line) and maximum acute stress-induced (shown as circles, solid line) levels of cort in 6-week-old captive tufted puffin chicks; means \pm SE.

reduced cort secretion in response to a short-term food deprivation and in response to a prolonged severe restriction (by 70% of ad libitum feeding regime) in metabolizable energy. We found that baseline and acute stress-induced cort levels appeared to increase in a continuous manner with an increase of endogenous lipid reserves of a chick. However, it was not clear from the results of this study if these increases in cort secretion were direct responses to an increase in daily energy intake or merely reflected a range of ‘developmental ages’ induced by dietary treatments (see discussion below).

Were puffin chicks food-stressed?

First, we need to establish whether tufted puffin chicks were in fact nutritionally stressed in our study. Decreases in growth of body mass, skeletal elements, low endogenous energy reserves, and hypothyroid function are most frequently used to assess nutritional stress in birds (see Introduction). Accordingly, tufted puffins fed reduced diets in this study had lower body mass and endogenous fat reserves, smaller wing chord, and decreased levels of thyroid hormones compared to birds fed ad libitum. However, all food-restricted birds were still growing their body mass and skeletal elements, albeit at low rates (Romano, 2000), and eventually would reach morphological development adequate for fledging (Kitaysky, 1999). This is a typical developmental pattern of food-restricted tufted puffins and their con-generics (Atlantic puffin *Fratercula arctica* (Wehle, 1980), horned puffin *Fratercula corniculata* (Piatt and Kitaysky, 2002b), and rhinoceros auklet *Cerorhinca monocerata* (Wehle, 1980)), which are also capable of slowing their growth rates in response to low rates of food intake. However, other alcids (e.g., common murre, *Uria aalge*) and gulls (e.g., black- and red-legged kittiwakes) nearly arrest their development if exposed to a similar range of dietary restrictions (Benowitz-Fredericks and Kitaysky, in press; Kitaysky et al., 1999, 2001a; Romano, 2000). Interestingly, an increased secretion of corticosterone in response to food shortages has been documented in chicks of these species.

Two lines of arguments based on the results of this experiment suggest that food shortage is an anticipated event in a tufted puffin chick’s life, and that chicks are well prepared for this. The first is that food-related stress usually affects thyroid hormone metabolism (deiodination of T4) in young birds selected for high growth rates: plasma T4 titers increase, whereas T3 titers decrease (e.g., in domestic chicken, Geris et al., 1999; reviewed in Kuhn et al., 1998). In other birds, such as the king penguin, *Aptenodytes patagonicus*, where low food intake of chicks is an anticipated event, plasma levels of the both (T4 and T3) hormones decline simultaneously during a prolonged period of winter fast, and these declines in both thyroid hormones can be related to energy saving mechanisms (Cherel et al., 2004). Thyroid hormones are known to be important growth

promoters in birds (reviewed in McNabb and King, 1993; McNabb et al., 1998). Hypothyroidism results in pronounced growth retardation in a wide variety of vertebrates (reviewed in Hulbert, 2000). In this study we also found that tufted puffin chicks simultaneously decrease T4 and T3 titers in response to a short-term food deprivation (when their endogenous energy reserves are not depleted) and prolonged nutritional deficits (when their endogenous energy reserves have been depleted). However, T3/T4 ratio, as an indirect measure of thyroid metabolism, did not change in response to a short-term fasting and prolonged nutritional deficits. Puffin chicks are capable of accumulating enormous fat reserves when food is abundant, decreasing their standard metabolic rates by ~50% in response to a short-term food deprivation, and adjusting their developmental rates to the rate of food provisioning (Kitaysky, 1999). A dramatic drop in thyroid hormone concentrations in fasting and severely food-restricted puffins in this study can be related to or is an essential part of this previously described physiological adaptation for intermittent food provisioning in puffins. Although it is currently unknown, a decrease in thyroid hormone production may be a direct mechanism for slowing growth of puffin chicks. However, if a decrease in deiodination of T4 is a reliable marker for nutritional stress in young birds, food-restricted Tufted puffins in this study were not physiologically stressed.

The second line of arguments is based on the observed reciprocal relationship between the hypothalamus–pituitary–thyroid (HPT) axis and HPA-axis activities during post-embryonic development in nutritionally stressed young chickens (e.g., Darras et al., 1996; Geris et al., 1999). Specifically, results of experimental studies suggest that either T3 is a negative modulator of the adrenal steroidogenic function (Carsia et al., 1997) or that elevated cort induces a decrease in the thyroid stimulating hormone (Geris et al., 1999), which in turn affects secretion of thyroid hormones (Darras et al., 1996). Although the exact causal mechanisms are unknown, a reciprocal relationship between cort and thyroid hormones concentrations is expected for young birds experiencing nutritional stress. Yet we found that plasma concentrations of cort and thyroid hormones declined in unison in fasting and the most food-restricted tufted puffin chicks. This result suggests that (a) HPT- and HPA-axes do not exhibit reciprocal relationships during nutritional deficits in all avian species, (b) a decrease in plasma concentrations of cort and thyroid hormones might be required to facilitate a switch in development of puffins from a high rate when food is abundant to a low rate when food becomes scarce, and (c) food-restricted Tufted puffin chicks were not physiologically stressed in this study.

To summarize, food-restricted tufted puffin chicks in this study were no doubt nutritionally limited and switched to a slow mode of development. However, they responded to both short- and long-term nutritional deficits as to a predictable/anticipated event during their development in the nest, and activated a physiological mechanism(s) that

would allow them to grow successfully despite severe food shortages.

Role of corticosterone in development

The adrenocortical stress response is a major mechanism allowing animals to deal with unpredictable changes in environmental conditions (mostly mammalian literature reviewed in Sapolsky et al., 2000). As we argue above, temporary food deprivation and low rates of food delivery by parents is an anticipated event in a puffin chick's life. Then why do puffins modulate their cort levels and maintain the adrenocortical stress response during development in the first place?

Glucocorticoids are known to be important chemical messengers in orchestrating numerous physiological functions of developing chicks (reviewed in McNabb et al., 1998), but glucocorticoids also cause a depletion of endogenous lipid reserves over their entire range of plasma concentrations (mostly mammalian literature reviewed in Sapolsky et al., 2000). It might be beneficial for fasting/food-limited puffin chicks to reduce baseline corticosterone secretion, which, in association with a reduced metabolic rate, would allow them to spare endogenous fat stores during food shortages.

The question remains as to why puffin chicks fed ad libitum have a tendency to increase their baseline and increased maximum acute stress-induced levels of cort (Fig. 2, 3, and 9). We believe that there is a hidden factor in this study that may be playing a role. Specifically, increased levels of cort have been suggested to occur prior to or at fledging in birds (Heath and Dufty, 1998). By the end of this study, tufted puffin chicks were at the same chronological age but certainly were at different stages of their morphological development, and chicks fed ad libitum were approaching the fledging age. In other words, maturation of tufted puffin chicks is tied to growth rather than to age (sensu; Schew and Ricklefs, 1998). Dry content of body tissues and water retention, as proxies for tissue maturation processes, provide further indirect evidence for that. Specifically, body tissues of younger chicks retain more water compared to those in older individuals, which is associated with the tissue maturation during development (reviewed in Ricklefs et al., 1998). In our study we found that food-restricted and thus slowly growing chicks had higher content of water in body tissues compared to ad libitum fed individuals (Fig. 8). Elevated baseline and acute stress-induced levels in relatively mature chicks fed ad libitum then might indicate that they were closer to fledging age compared to food-restricted individuals. Higher levels of cort in wild chicks may also be associated with them being somewhat older than captive chicks and therefore even closer to fledging than ad libitum fed captive individuals. Although this explanation for the elevated levels of cort in ad libitum fed and wild individuals in our study seems plausible, the ontogenetic changes of the HPA

function in food-restricted puffins should be addressed in a specifically designed experiment.

Costs of HPA axis suppression

Another open question is whether there is a cost of the suppression of adrenocortical function in food-limited puffin chicks (apparent in the reduced maximum cort secretion in response to the handling stressor). Typically, tufted puffins nest in earthen burrows and their chicks are relatively safe in the nest (Piatt and Kitaysky, 2002a). However, they are still vulnerable to predation by gulls and ravens while waiting for their parents at the burrow entrance. Whether a slow-developing chick with decreased activity of the HPA-axis is less vigilant and less capable of escaping predators than its well-fed counterparts is not known, but if this is the case, it might be one of the behavior-mediated costs of the suppression of adrenocortical response. Field observations of high depredation of hungry puffin chicks by gulls provide indirect evidence for this (S. Hatch and authors' unpublished observations). Also, a puffin chick's ability to mount an adrenocortical response might be beneficial during agonistic behavioral interactions among chicks in neighborhoods. Tufted puffin parents drop food delivered for their chick on the ground close to the burrow entrance and do not discriminate their chick from other puffin chicks (Piatt and Kitaysky, 2002a,b; Wehle, 1980). Burrows are often connected by underground tunnels, which would allow chicks to move among borrows and steal food from their neighbors. Puffin chicks are extremely aggressive toward each other, which might serve as a mechanism preventing chicks from stealing food. Increased cort secretion facilitates aggression in other nest-bound seabird chicks (Kitaysky et al., 2003), and results of our recent experimental study suggest that the responsiveness of HPA-axis to external stressors might be involved in regulation of aggressive interactions between Tufted puffin chicks (Kitaysky et al., unpublished). Thus, the adrenocortical response of tufted puffin chicks to external stressors might be required to maintain vigilance and territorial aggression, and individuals with a dampened HPA-axis sensitivity would be at a disadvantage.

Mechanisms of HPA axis suppression

Chronic elevation of corticosterone during early development can have long-term deleterious effects on seabirds (Kitaysky et al., 2003). We observed a reduction in cort levels in puffin chicks in response to short- and long-term nutritional deficits. This physiological adaptation may allow a chick to extend its development in the nest, while eluding detrimental effects of chronic cort elevation. Mechanistically, this could be a result of suppressed secretion or increased clearance rate. First, we define the suppression as following: cort secretion is actively suppressed in response to food restriction, so that more severe food restriction

results in a greater suppression of cort secretion. We believe that we did find direct evidence for the suppression of cort secretion at least in the case of the adrenocortical response of puffin chicks to acute stress of handling and restraint. The more restricted chicks were—the less cort they secreted during the stress procedure. Thus compared to their cohorts on less restricted diets, their cort secretion was suppressed. Also, baseline and maximum levels of cort were strongly positively correlated (Fig. 6). This linear relationship, with a slope equals 1, provides further albeit indirect evidence that the secretion of cort was suppressed in food-limited puffin chicks.

Suppression may be the result of reduced adrenal capacity or adrenal sensitivity (e.g., Rees et al., 1985; Romero et al., 1998) of food-restricted puffins. Injection of ACTH induced a robust twofold increase in plasma cort compared to maximum acute stress-induced levels in wild tufted puffin chicks, and ACTH-induced cort concentrations were similar to maximum acute stress-induced levels observed in adult tufted puffins (Kitaysky, unpublished data). This suggests that the adrenal sensitivity/capacity of a developing puffin chick is not suppressed and a mechanism upstream from the adrenals blunts corticosterone release. Whether production/secretion of corticotropin-releasing factor (CRF) or arginine-vasotocin (AVT, also strong ACTH secretagogue) is decreased during development, or the sensitivity of the pituitary to the signal from the hypothalamus is down-regulated, is not known and requires further investigation. The results of this current study allow us to make testable predictions. For instance, reduced daily food intake also resulted in a decrease in daily water consumption of puffin chicks, which rely solely on water from food. Nevertheless, food-limited individuals were not dehydrated (e.g., see Fig. 8), suggesting that a mechanism of water retention was activated. Increase in AVT secretion is expected in water-limited animals (Norris, 1996). Increase in AVT elicits cort release in birds (Romero et al., 1998) probably via either enhancing effects of CRF effects on ACTH production by the pituitary, or acting independently (Tachibana et al., 2004). Negative feedback of high cort on ACTH secretion would be prevented as AVT released from the posterior pituitary into the blood in large quantities to facilitate water retention in water-limited individuals would eventually reach the anterior pituitary and act as the primary or secondary secretagogue for ACTH. In accordance with this scenario, increases in baseline cort secretion have been observed in food- and water-restricted chicks of several seabird species (see Introduction). Yet, tufted puffin chicks respond to food/water limitations by decreasing baseline cort. We might therefore predict that in a developing tufted puffin ACTH release at the pituitary level is AVT-independent, which would be easy to test.

The second possibility is that in addition to reduced cort secretion as discussed above, the clearance rate of plasma cort could be higher among food-restricted chicks (although this would involve an increased hepatic function

and down-regulation of cort receptors (Breuner and Orchnik, 2001), two mechanisms that require an increase in metabolic rate in food-limited chicks, which does not occur in the tufted puffin). A decrease in plasma globulin's binding capacity for cort may also result in more free cort, which could both facilitate its clearance rates and increase negative feedback on further cort secretion (Breuner and Orchnik, 2002). Results of recent studies in birds suggest that a decrease in cort-binding globulins may also increase a ratio of free to bound hormone, which would potentially enhance cort metabolic and genomic effects (Love et al., 2004; Lynn et al., 2003). Whether this is the case in the tufted puffin is currently unknown and requires further investigation.

Wild versus captive puffin chicks

It is possible that the variations in adrenal response of tufted puffin chicks observed in this study resulted from conditions of captivity rather than a functional response to variations in energy content and quality of food. The adrenocortical response of wild puffin chicks to a standardized acute stress of handling and restraint was indeed higher than that in captive individuals. However, baseline levels of cort (a parameter that is not known to be affected by frequent handling) were also higher in wild chicks compared to those in captives. Furthermore, baseline and maximum acute stress-induced levels of cort were strongly affected by dietary treatments and correlated with endogenous fat reserves of captive individuals, and captive chicks in all treatments were subject to similar controlled conditions. The only differences between them were the energy content and nutritional quality of their food. In addition, other studies of the adrenal response in young birds to a standardized acute stressor did not indicate that those birds habituated to frequent handling (Dufty and Belthoff, 1997; Freeman et al., 1981; Heath and Dufty, 1998; Kitaysky et al., 1999, 2001a). Thus, we conclude that the results of this study demonstrate differences in the functional responses of tufted puffin chicks to variations in energetic content and nutritional quality of their food.

Finally, in this study nutritional history of puffin chicks prior to taking them in captivity was not known. One might argue that the suppression of HPA activity in response to nutritional deficits may reflect their exposure to food shortages during the early neonatal period. For instance, the adrenocortical stress response of 36-day-old chickens to food deprivation was dampened by their exposure to a moderate food-restriction earlier in life (at 60% of ad libitum during 4–6 days post-hatch) (Zulkifli et al., 1995). However, results of our study suggest that it is not the case. Specifically, wild puffin chicks, which were fed by their parents and likely experienced intermittent energy provisioning during development, had higher baseline and acute stress-induced cort compared to captive individuals.

Conclusion

This is the first experimental study showing a reduction of the HPA-axis activity in response to nutritional deficits in wild birds. This mechanism may serve as an anti-stress physiological adaptation against high variability of foraging resources in birds. We do not know how widespread a phenomenon is. It is expected to occur in other systems where long-lived parents are reluctant to buffer their current young from fluctuations in food supply.

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